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Shed Syndecan-1 Translocates to the Nucleus of Cells Delivering Growth Factors and Inhibiting Histone Acetylation

*A NOVEL MECHANISM OF TUMOR-HOST CROSS-TALK**

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Background: Shed syndecan-1 enhances tumor growth and progression.
Results: Syndecan-1 shed by myeloma cells translocates to the nucleus of cells where it delivers growth factors and inhibits histone acetylation.
Conclusion: Shed syndecan-1 facilitates tumor-host cross-talk by translocating to the nucleus of host cells.
Significance: Nuclear translocation of shed syndecan-1 has widespread functional implications in cancer and other diseases where syndecan-1 shedding is elevated.

The heparan sulfate proteoglycan syndecan-1 is proteolytically shed from the surface of multiple myeloma cells and is abundant in the bone marrow microenvironment where it promotes tumor growth, angiogenesis, and metastasis. In this study, we demonstrate for the first time that shed syndecan-1 present in the medium conditioned by tumor cells is taken up by bone marrow-derived stromal cells and transported to the nucleus. Translocation of shed syndecan-1 (sSDC1) to the nucleus was blocked by addition of exogenous heparin or heparan sulfate, pretreatment of conditioned medium with heparinase III, or growth of cells in sodium chlorate, indicating that sulfated heparan sulfate chains are required for nuclear translocation. Interestingly, cargo bound to sSDC1 heparan sulfate chains (*i.e.* hepatocyte growth factor) was transported to the nucleus along with sSDC1, and removal of heparan sulfate-bound cargo from sSDC1 abolished its translocation to the nucleus. Once in the nucleus, sSDC1 binds to the histone acetyltransferase enzyme p300, and histone acetyltransferase activity and histone acetylation are diminished. These findings reveal a novel function for shed syndecan-1 in mediating tumor-host cross-talk by shuttling growth factors to the nucleus and by altering histone acetylation in host cells. In addition, this work has broad implications beyond myeloma because shed syndecan-1 is present in high levels in many tumor types as well as in other disease states.

Syndecan-1 is a cell surface heparan sulfate proteoglycan known to participate in adhesion, motility, invasion, and intracellular signaling (1). Many of these diverse functions result from heparan sulfate chain binding to a variety of factors such as growth factors, chemokines, and cytokines. In addition, it has been shown that the core protein of syndecan-1 can facilitate intracellular signaling and also directly interact with integrins at the cell surface (2). However, syndecan-1 localization and function are not restricted solely to the cell surface. Syndecan-1 can be proteolytically cleaved or shed from the surface of cells yielding the syndecan-1 ectodomain with covalently attached heparan sulfate chains (3). Thus, sSDC1[2] is capable of presenting heparin-binding factors to other cells or sequestering their cargo within the extracellular matrix (4). Our laboratory has shown that sSDC1 is present at high levels in some myeloma patients, and this was found to be predictive of poor prognosis (5, 6). *In vivo* models of myeloma reveal that elevated sSDC1 enhances growth, angiogenesis, and metastasis of tumor cells (7, 8). In addition, treatment of myeloma cells with commonly used anti-myeloma drugs stimulates syndecan-1 shedding, a potentially negative impact of therapy (9).

Syndecan-1 has been found in the nucleus of both myeloma and mesothelioma cells (10, 11), and we previously demonstrated that loss of syndecan-1 from the nucleus of myeloma cells resulted in an increase in HAT activity and led to increased expression of genes that drive tumor progression (12). Although a portion of syndecan-1 in the nucleus is the full-length form of the molecule containing the ectodomain, transmembrane, and cytoplasmic domains, it was not known whether the shed form of the proteoglycan could translocate to the nucleus. Exogenously added heparan sulfate chains can translocate to the nucleus (13), raising the possibility that the syndecan-1 ectodomain with its heparan sulfate chains could enter the nucleus. In this study, we demonstrate for the first time that sSDC1 translocates to the nucleus of both tumor cells and bone marrow-derived stromal cells where it delivers growth factors and inhibits HAT activity and histone acetylation. This work reveals a novel function of sSDC1 and extends our understanding of how sSDC1 facilitates communication within the tumor microenvironment.

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[2] The abbreviations used are: sSDC1, shed syndecan-1; HAT, histone acetyltransferase; HGF, hepatocyte growth factor; Hep III, heparinase III.

EXPERIMENTAL PROCEDURES

Cell Lines and Transfections—CAG cells were established from the bone marrow aspirate of a patient with myeloma at the Arkansas Cancer Research Center as described previously (14). ARH-77 cells were obtained from the American Type Culture Collection. Hamster ovary CHO-pgsA-745 cells were provided by Dr. Jeffrey Esko, University of California at San Diego. Stromal ST2 cells derived from murine bone marrow were kindly provided by Dr. Thomas Clemens, Johns Hopkins University. All cell lines were grown in RPMI 1640 growth medium supplemented with 10% fetal bovine serum. CAG cells were stably transfected with cDNA encoding the region for the extracellular portion of the human syndecan-1 core protein (amino acids 1–252) in the pcDNA3 vector (sSDC1 construct). ARH-77 cells, which lack syndecan-1 expression (15), were stably transfected with an sSDC1 construct bearing mutated glycosaminoglycan attachment sites (CAG ΔGAG) as described previously (16).

Western Blotting—Cells in suspension culture were pelleted by centrifugation and washed twice with ice-cold PBS before cell lysis. Cells growing in monolayers were rinsed twice with ice-cold PBS and lysed directly on the plate. For preparing whole cell lysates, cells were incubated in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 0.5% Triton X-100) containing 1× HALT protease and phosphatase inhibitor mixture (Pierce) and incubated on ice for 30 min. Lysates were centrifuged at 12,000 × *g* at 4 °C for 15 min, and the supernatants were removed from the pellets. For non-nuclear and nuclear protein extraction, cells were incubated in a hypotonic buffer (20 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$) supplemented with 1× HALT protease and phosphatase inhibitor mixture and incubated on ice for 10 min. Nonidet P-40 (10%) was added to the homogenate, and the mixture was vortexed for 10 s at the highest setting. The homogenate was centrifuged for 10 min at 3000 rpm at 4 °C. The supernatant (non-nuclear fraction) was transferred to a clean microcentrifuge tube. The remaining pellet was resuspended in lysis buffer on ice for 40 min. Every 10 min, the nuclear homogenate was vortexed for 10 s at the highest setting. The nuclear homogenate was centrifuged at 4 °C at 12,000 rpm for 10 min. Supernatant (nuclear fraction) was transferred to a clean microcentrifuge tube. Proteins isolated in the supernatant were quantified using the BCA protein assay reagent kit (Pierce). Equal amounts of protein were loaded onto 4–20% gradient SDS-polyacrylamide gels (Bio-Rad), transferred to a positively charge nylon membrane (Nytran SPC: Schleicher & Schuell), and probed with the respective antibody followed by horseradish peroxidase-conjugated secondary antibody. Antibodies utilized in this study were goat anti-human syndecan-1 (R&D Systems), histone H3 (Millipore), acetylated histone H3 (Millipore), lamin A/C (Cell Signaling), p300 (Millipore), and GAPDH (Cell Signaling). Immunoreactive bands were detected using enhanced chemiluminescence (GE Healthcare).

Immunocytochemistry and Confocal Imaging—Cells growing in monolayers were seeded onto D-lysine-coated coverslips (BD Biosciences) for 24 h. Cells were fixed with 4% formaldehyde in PBS for 30 min at room temperature. For cells in suspension culture, the cells were loaded into the cytospin funnel and spun onto a slide at 1000 rpm for 5 min. Cells were permeabilized with 0.5% Triton X-100 in PBS for 5 min at room temperature and rinsed with PBS. The slides were incubated with 1% BSA in PBS for 30 min at room temperature followed by incubation with anti-syndecan-1 (1:100 in 1% BSA in PBS) overnight at 4 °C. After washing, the cells were incubated with anti-goat-Alexa Fluor 488 (1:200; Invitrogen) at room temperature for 1 h. After washing in PBS, cells were stained with DAPI and mounted for viewing. Cells were viewed and photographed using a Nikon A1 confocal laser-scanning microscope. The NIS-Elements software provided by Nikon was used to visualize and separate Z-stack images.

Preparation and Treatment of Conditioned Media—The CAG sSDC1-transfected cells were seeded at a concentration of 5×10^5 cells/ml in complete growth medium and incubated for 48 h at 37 °C and 5% CO_2 in a humidified chamber. Media conditioned by these cells were removed at the end of the incubation period and centrifuged at 1000 rpm twice to remove all cells. The clarified media were then aliquoted and stored at −20 °C until further use. For experiments requiring the removal of heparan sulfate chains from syndecan-1 core protein, 10 μg/ml heparinase III enzyme (generously provided by Dr. Jian Liu, University of North Carolina at Chapel Hill) was added to conditioned medium for 4 h at 37 °C. For experiments involving heparin or heparan sulfate, 10 μg/ml was added to conditioned medium prior to its addition to cell cultures. For chlorate experiments, cells were grown in the presence of 20 mM sodium chlorate or as a control, 20 mM sodium chlorate, and 10 mM sodium sulfate for 48 h prior to collection of conditioned medium as described previously (17). Experiments also included depletion of heparin-binding growth factors, such as HGF, from the conditioned media by incubating overnight at 4 °C with 25 μl of heparin immobilized on agarose (MP Biomedicals). Co-immunoprecipitation of sSDC1 and bound factors from nuclear lysate was performed by using 2 μg/ml anti-human syndecan-1 or isotype-specific control antibody bound to protein G-Sepharose beads as described previously (18).

Purification of Syndecan-1 Ectodomain—Conditioned medium from CAG cells stably expressing cDNA encoding the region for the extracellular portion of the human syndecan-1 core protein was collected, and 2 M urea and 50 mM sodium acetate, pH 4.5, were added. The medium was incubated with DEAE-Sepharose overnight at 4 °C. Beads were collected by centrifugation and washed in PBS. Syndecan-1 ectodomain was eluted with 1 M NaCl; the buffer was exchanged, and the sample was concentrated. The amount of partially purified sSDC1 was measured by ELISA (19).

Syndecan-1 and HGF Quantification—Levels of sSDC1 in the conditioned media or nuclear lysate were assessed by enzyme-linked immunosorbent assay (ELISA) using an Eli-pair kit specific for human syndecan-1 core protein (Cell Sciences). The standard curve was linear between 8 and 256 ng/ml, and all samples were diluted to concentrations within that range. Levels of HGF in the conditioned media or nuclear lysate were measured using a human HGF ELISA (RayBiotech) following the manufacturer's protocol. All samples were run in duplicate.

Histone Acetyltransferase Activity Assay—Conditioned medium containing sSDC1 was added to cells for 6 h at 37 °C. The

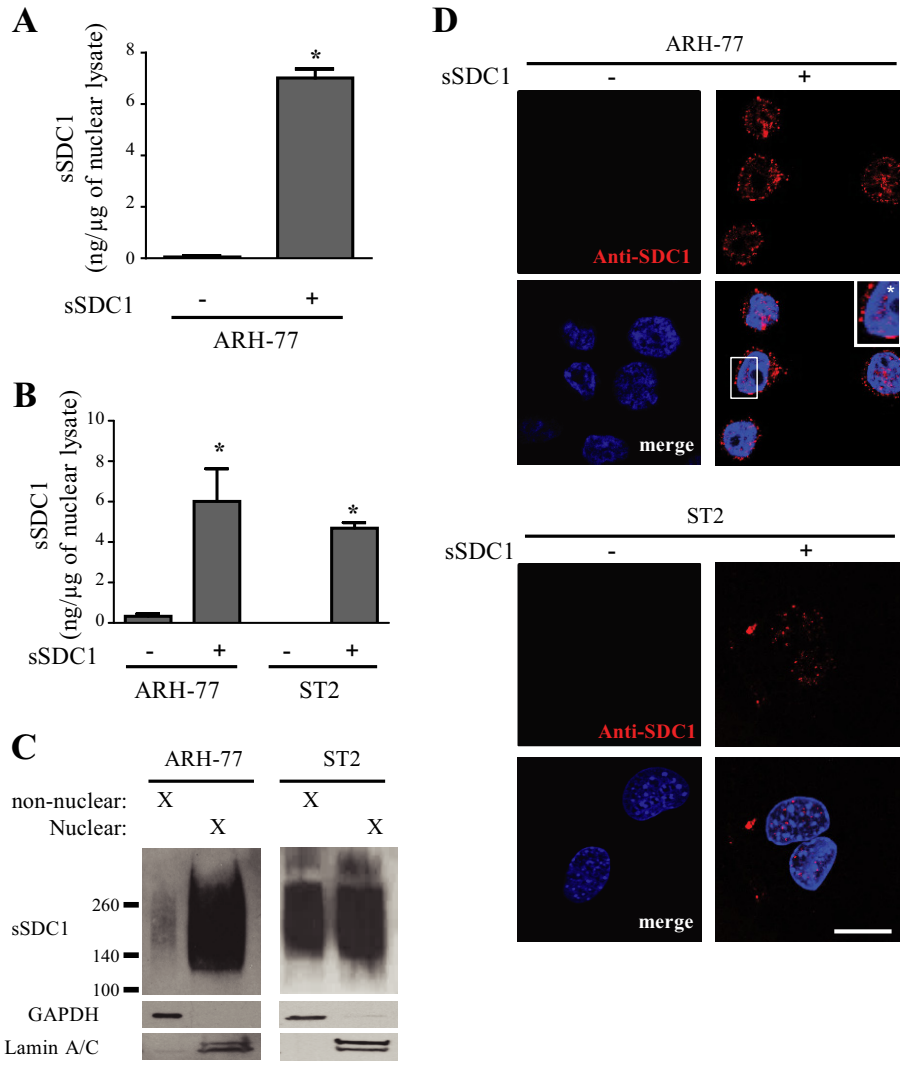

FIGURE 1. **Shed syndecan-1 translocates to the nucleus of cells.** *A,* conditioned medium from heparanase-high CAG cells containing sSDC1 was added to ARH-77 cells (+). As a control, ARH-77 cells were incubated with medium lacking sSDC1 (−). After incubation with conditioned media for 1 h, ARH-77 cells were collected and fractionated to separate non-nuclear and nuclear proteins. Nuclear lysates were analyzed for their level of sSDC1 by ELISA. *, $p < 0.05$ *versus* untreated control. *B,* medium without sSDC1 (−) or with sSDC1 (+) from cells engineered to secrete high levels of syndecan-1 ectodomain was incubated with ARH-77 or ST2 cells for 1 h. Nuclear lysates were analyzed for their level of sSDC1 by ELISA. Data are mean ± S.E. of three independent experiments. *, $p < 0.05$ *versus* untreated controls for each individual cell line. *C,* cell lysates utilized for sSDC1 ELISA measurements were further analyzed by immunoblotting for syndecan-1. GAPDH (non-nuclear control) and lamin A/C (nuclear control) were probed in the same blot to demonstrate separation of the two cellular fractions. *D,* conditioned medium without (−) or with (+) sSDC1 was incubated with ARH-77 or ST2 cells and immunostained for syndecan-1 (*red*), and nuclei were counterstained with DAPI (*blue*). Confocal images depict a *z*-plane through the center of the cell nucleus. *Scale bar*, 10 μm. *, enlarged from the *boxed area* in the same panel to demonstrate localization of sSDC1 within areas of the nucleus that stain poorly with DAPI (*i.e.* areas of euchromatin).

nuclear lysate was collected, and HAT activity was measured using a fluorometric assay kit (Biovision) following the manufacturer's protocol. All samples were run in duplicate.

Statistical Analysis—Experiments were repeated a minimum of three times. Comparisons were analyzed by Student's *t* test using GraphPad Prism. *p* values less than 0.05 were considered statistically significant. All data are mean plus or minus standard error of the mean (S.E.).

RESULTS

Shed Syndecan-1 Is Transported to the Nucleus of Cells—To determine whether sSDC1 translocates to the nucleus, we first utilized human CAG myeloma cells that have high levels of heparanase expression and shed high levels of syndecan-1 (20). When medium collected from these cells was incubated with ARH-77 cells, a human lymphoblastoid cell line lacking endogenous expression of syndecan-1 (15), an ELISA of nuclear lysates revealed that a large amount of syndecan-1 had reached the nucleus (Fig. 1*A*). To facilitate the analysis further, we utilized conditioned medium from human myeloma CAG cells that were engineered to stably express a form of syndecan-1 lacking its transmembrane and cytoplasmic domain. These cells secrete high levels of syndecan-1 that mimics the proteolytically shed form of the molecule (we will refer to this as shed syndecan-1 (sSDC1)). When medium conditioned by these cells was incubated with ARH-77 cells, high levels of nuclear sSDC1 were detected (Fig. 1*B*). Similarly, the human sSDC1 also translocated to the nucleus of ST2 cells, a murine bone marrow-derived stromal cell line. Interestingly, the human sSDC1 was detected in the nucleus by ELISA as early as 15 min

after addition to cells indicating that the translocation of sSDC1 to the nucleus is a relatively rapid process.[3] Western blots confirmed the presence of high levels of sSDC1 in the nuclear fractions of ARH-77 and ST2 cells (Fig. 1*C*). Non-nuclear and nuclear lysates were collected by fractionation, and lamin A/C and GAPDH were used to show fraction fidelity between the nuclear and non-nuclear proteins, respectively. Western blots using anti-human syndecan-1 antibody demonstrate that the shed form of the proteoglycan can be transported to the nucleus of these human and murine cells (Fig. 1*C*). Interestingly, the stromal cells also show high levels of sSDC1 in the non-nuclear fraction as compared with the ARH-77 cells (Fig. 1*C*, *right blot*). This is due to sSDC1 binding to the extracellular matrix produced by these stromal cells.[3]

To further confirm the presence of sSDC1 in the nucleus, immunocytochemistry and confocal microscopy were employed. ARH-77 or stromal cells were incubated in medium containing sSDC1, fixed, and then stained with fluorescently tagged anti-human syndecan-1 antibody. Confocal images confirmed the presence of syndecan-1 in the nucleus of both the cell types (Fig. 1*D*). Moreover, most of the nuclear syndecan-1 is localized in discrete patches within euchromatin indicating sSDC1 specifically localizes to areas of active gene transcription where it may serve a regulatory function.

Shed Syndecan-1 Suppresses HAT Activity and Binds to HAT Protein p300 in the Nucleus—Because loss of syndecan-1 from the nucleus of myeloma cells resulted in enhanced HAT activity (12), we asked whether sSDC1 delivered to the nucleus of the ST2 bone marrow cells would diminish HAT activity. When ST2 cells were exposed to sSDC1, both the level of acetylated histone H3 and HAT activity within the nucleus were decreased (Fig. 2, *A* and *B*). This decrease in acetylated histone and HAT activity was abolished by pretreatment of the medium containing sSDC1 with heparinase III (hep III), a bacterial enzyme that extensively degrades heparan sulfate chains. To determine whether this inhibition of HAT activity could be due to a direct interaction between syndecan-1 and HAT enzymes, ST2 cells that were preincubated with sSDC1 were lysed; their nuclear fractions were prepared, and syndecan-1 was removed by immunoprecipitation. Western blotting revealed that the HAT enzyme p300 co-immunoprecipitated with syndecan-1 (Fig. 2*C*). Pretreatment of the nuclear extract with hep III prior to immunoprecipitation resulted in the inability of p300 to immunoprecipitate with syndecan-1 indicating that p300 is binding to the heparan sulfate chains on the syndecan-1 core protein. Interestingly, immunofluorescence images of p300 reveal a speckled pattern in the nucleus similar to the nuclear staining of shed syndecan-1 (21, 22). Together, these experiments indicate that sSDC1 in the nucleus of ST2 cells binds to the HAT enzyme p300, and this is accompanied by diminished HAT activity and decreased acetylation of histone H3.

Heparan Sulfate Chains of Shed Syndecan-1 Are Required for Translocation of the Proteoglycan to the Nucleus—To determine whether the heparan sulfate chains of syndecan-1 are required for its translocation to the nucleus, medium containing sSDC1 was either pretreated with hep III or excess heparan sulfate or heparin were added to the medium prior to its incubation with ST2 cells. Under all three conditions, translocation of the sSDC1 to the nucleus was inhibited, indicating that heparan sulfate chains are required for this process (Fig. 3*A*). These findings were further confirmed by immunocytochemistry and confocal imaging (Fig. 3*B*). Immunocytochemistry of ST2 cells incubated in medium conditioned by cells that produce sSDC1 core protein lacking heparan sulfate chains (sSDC1 ΔGAG) also confirmed the requirement of heparan sulfates for transport of sSDC1 to the nucleus (Fig. 3*B*). To determine whether sulfation

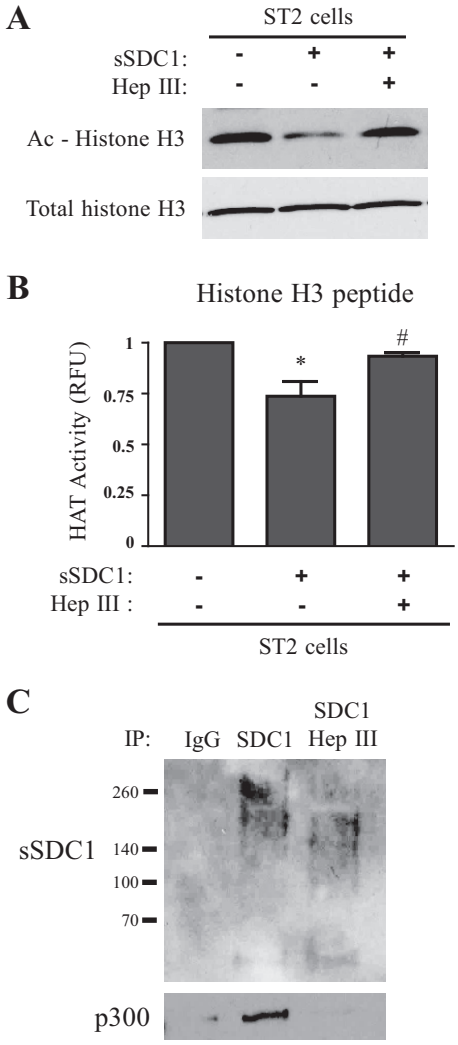

FIGURE 2. **Shed syndecan-1 down-regulates histone acetyltransferase activity and histone H3 acetylation.** *A,* ST2 cells incubated for 6 h with conditioned medium containing sSDC1 diminished the level of acetylated histone H3. This effect was prevented by pretreatment of medium with bacterial heparinase III (*Hep III*). Cell lysates were analyzed by Western blot for acetylated histone H3 or total histone H3. *B,* in experiments similar to those in *A*, histone acetyltransferase activity was quantified by fluorimetric activity assay. *RFU*, relative fluorescence units. *, $p < 0.05$ *versus* untreated controls; #, not significant *versus* untreated control. *C,* sSDC1 was immunoprecipitated (*IP*) from nuclear extracts and probed by Western blot for syndecan-1 and p300 (*middle lane*). The p300 co-immunoprecipitated with sSDC1. Pretreatment of extracts with hep III followed by sSDC1 immunoprecipitation failed to co-immunoprecipitate p300 (*right lane*). Note that the size of syndecan-1 is diminished following hep III treatment (*right lane*) confirming that the enzyme is cleaving the heparan sulfate chains, although the chondroitin sulfate chains remain attached to the core protein.

[3] M. D. Stewart and R. D. Sanderson, unpublished observation.

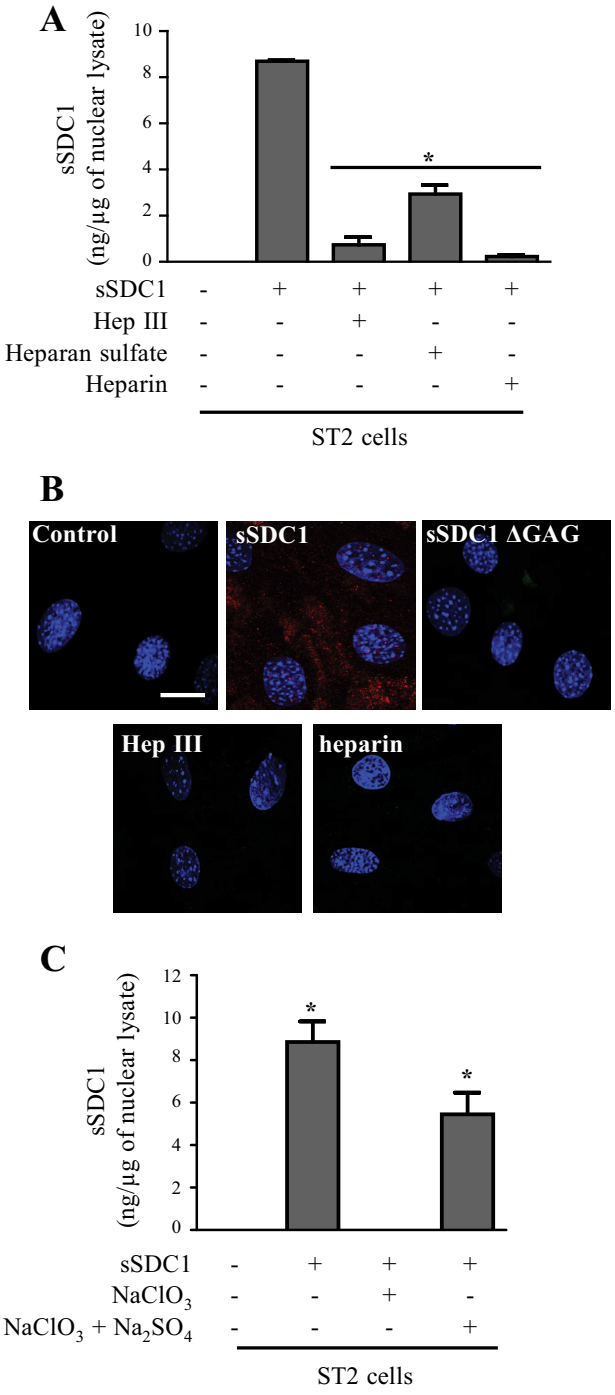

FIGURE 3. **Intact heparan sulfate chains are required for shed syndecan-1 to translocate to the nucleus of cells.** *A,* sSDC1-containing medium was added to ST2 cells without or with prior treatment of medium with hep III for 2 h, or the conditioned medium was added in the presence of excess heparan sulfate (10 μg/ml) or heparin (10 μg/ml). The amount of sSDC1 in the nucleus of ST2 cells was quantified by ELISA. Data are mean ± S.E. of three independent experiments. *, $p < 0.05$ *versus* untreated controls. *B,* following incubation with medium containing sSDC1, ST2 cells were immunostained for syndecan-1 (*red*). Results confirm ELISA data shown in *A*. Incubation of ST2 cells with conditioned medium collected from CAG cells expressing sSDC1 without glycosaminoglycan chains (sSDC1 ΔGAG) confirms heparan sulfate chains are required for sSDC1 translocation to the nucleus. The nuclei are counterstained with DAPI (*blue*). *Scale bar*, 10 μm. *C,* CAG cells engineered to express sSDC1 were grown in the presence of $NaClO_3$ or $NaClO_3$ + Na_2SO_4 for 48 h, and conditioned media were collected and syndecan-1 levels measured. Equal amounts of syndecan-1 were added to ST2 cells for 2 h; nuclear lysates were collected, and the level of sSDC1 in the nucleus was quantified by ELISA. Data are mean ± S.E. of three independent experiments. *, $p < 0.05$ *versus* untreated controls.

of heparan sulfate is necessary for sSDC1 translocation, cells were grown in medium containing sodium chlorate, which results in poorly sulfated heparan sulfate chains (23). As a control, cells were grown in medium containing both sodium chlorate and excess sodium sulfate, which negates the effect of sodium chlorate leaving the heparan sulfate chains sulfated. Conditioned medium containing equal amounts of sSDC1 were then added to ST2 stromal cells for 2 h, and nuclear lysate was collected, and syndecan-1 was quantified by ELISA. Results demonstrated that sSDC1 lacking sulfation was not translocated to the nucleus (Fig. 3*C*). This is consistent with the report that decreased sulfation diminishes the rapid uptake of modified heparins (24).

For Translocation of Shed Syndecan-1 to the Nucleus, Its Heparan Sulfate Chains Must Be Complexed with Heparin-binding Molecules—We have previously demonstrated that sSDC1 via its heparan sulfate chains binds to factors such as VEGF and HGF to potentiate their biological activity (8, 18). In addition, it is known that heparin or heparan sulfate can facilitate growth factor translocation to the nucleus (25). Because the ectodomain of syndecan-1 lacks a consensus nuclear translocation sequence, we speculated that factors bound to heparan sulfate on sSDC1 were mediating translocation of syndecan-1 to the nucleus. To test this, conditioned medium containing sSDC1 was incubated with heparin-agarose overnight to strip heparan sulfate-binding molecules like VEGF and HGF bound to the syndecan-1 heparan sulfate. To verify that this procedure could in fact strip the chains, we monitored levels of HGF, a heparan-binding growth factor abundantly secreted by myeloma cells and shown to be bound to the heparan sulfate chains of sSDC1 in the conditioned medium (18). Results revealed that heparin-agarose treatment efficiently removed HGF from the conditioned medium (Fig. 4*A*), without altering the level of sSDC1 as confirmed by ELISA.[3] The conditioned medium containing sSDC1 cleared of heparin-binding factors was added to the stromal cells for 2 h. Analysis of nuclear lysates from these cells revealed that sSDC1 was not present in the nucleus (Fig. 4*B*). To confirm these results, partially purified sSDC1 (having been stripped of heparin-binding factors during the purification process) was also added to the stromal cells for 2 h. Again nuclear lysates were collected from these cells, and no sSDC1 was detected by ELISA. To determine whether HGF was sufficient to promote sSDC1 translocation to the nucleus, we added recombinant HGF to the purified sSDC1. However, sSDC1 was still not translocated to the nucleus.[3] This indicates that heparin-binding factors other than, or in addition to, HGF must be present for translocation of sSDC1 to the nucleus.[3]

Shed Syndecan-1 Forms a Complex with HGF and Transports It to the Nucleus—We next investigated whether sSDC1 could shuttle heparan sulfate-binding molecules to the nucleus. The bone marrow microenvironment is replete with growth factors, in particular HGF which is the most highly expressed growth factor in myeloma (26, 27). To determine whether sSDC1 could shuttle HGF to the nucleus, we utilized the cell line CHO pgsA-745 that lacks xylotransferase activity and thus does not produce any glycosaminoglycans (28). This allowed us to track nuclear translocation of HGF solely due to the heparan sulfate chains of sSDC1. The glycosaminoglycan-deficient CHO cells

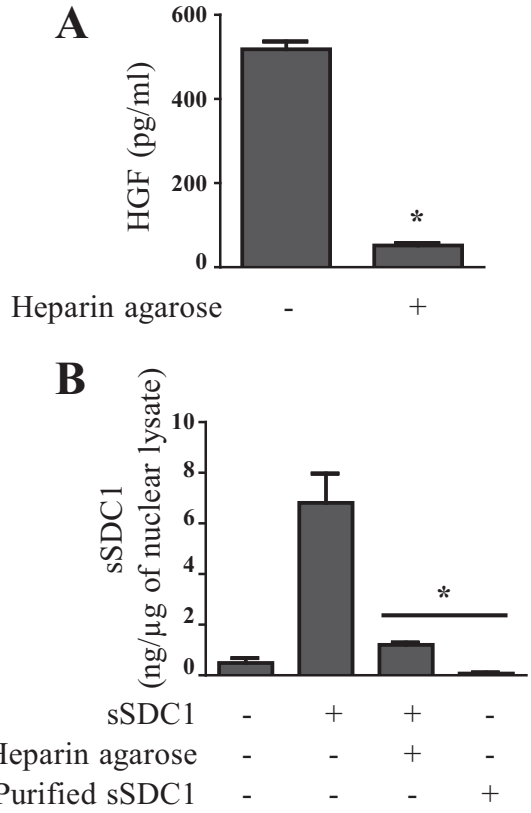

FIGURE 4. **Heparan sulfate-binding factors are required for shed syndecan-1 translocation to the nucleus.** *A,* conditioned medium containing sSDC1 was incubated with heparin-agarose beads overnight to strip heparin-binding growth factors from the heparan sulfate chains of syndecan-1. An ELISA was performed to measure levels of HGF after heparin-agarose treatment to confirm that the heparin-agarose beads had removed heparin-binding proteins. *B,* conditioned medium was pretreated with heparin-agarose and incubated with ST2 cells for 2 h, and the level of sSDC1 in the nucleus was assessed by ELISA. To confirm that heparin-binding factors are required for translocation to the nucleus, purified sSDC1 was incubated with ST2 cells for 2 h, and the level of sSDC1 in the nucleus was assessed by ELISA. All data are mean ± S.E. of three independent experiments. *, $p < 0.05$ *versus* untreated controls.

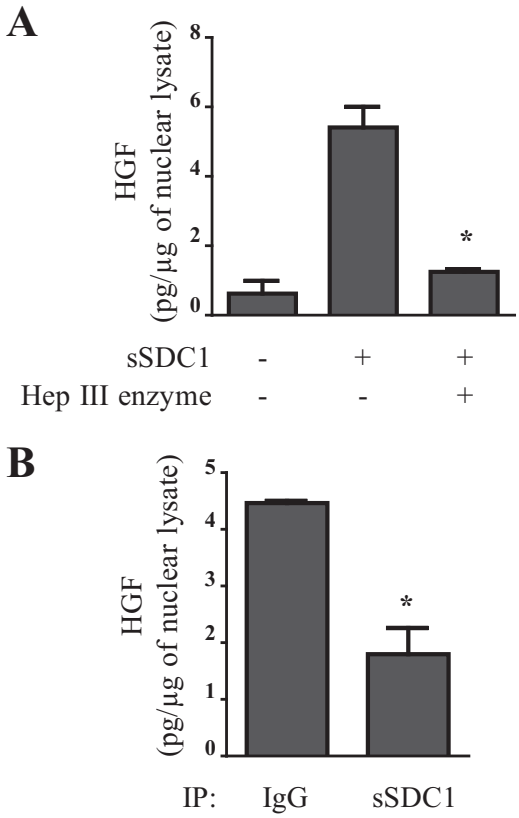

FIGURE 5. **Shed syndecan-1 shuttles HGF to the nucleus and syndecan-1·HGF exists as a complex within the nucleus.** *A,* conditioned medium containing high levels of sSDC1 and HGF was collected from myeloma cells and pretreated with hep III enzyme or added directly to CHO-pgsA745 cells. Nuclear lysates were collected, and an ELISA was performed to determine levels of HGF in the nucleus. *, $p < 0.05$ *versus* untreated controls. *B,* nuclear lysates were prepared from CHO-pgsA745 cells that had been incubated for 1 h in medium containing high levels of shed syndecan-1 and HGF. The level of HGF was determined with or without prior immunoprecipitation (*IP*) of syndecan-1 from the lysate.

were incubated for 1 h with conditioned medium from CAG heparanase-high cells. Medium from these cells contains high levels of both HGF and sSDC1 (18). Analysis of nuclear lysate revealed the presence of HGF in the nucleus, whereas pretreatment of the conditioned medium with hep III inhibited HGF transport to the nucleus (Fig. 5*A*). This indicates that the heparan sulfate chains of sSDC1 are required for HGF to translocate to the nucleus. To determine whether HGF remains bound to syndecan-1 within the nucleus, following a 1-h incubation with medium conditioned by the heparanase-high cells, the nuclear lysate from the CHO cells was isolated, and sSDC1 was immunoprecipitated. This removed ~60% of the HGF present in the nuclear lysate as compared with IgG isotype control (Fig. 5*B*). Together, these results indicate that a complex between sSDC1 and heparan sulfate-binding factors such as HGF is required for their translocation to the nucleus and that they remain as a complex once they reach the nucleus.

DISCUSSION

Cross-talk between tumor and host cells is critical to the establishment of a microenvironment conducive to tumor growth and progression. This cross-talk can be accomplished in many ways, for example by activation of cell surface signaling receptors or uptake of tumor-derived exosomes by the host cell (29). In this work, we have demonstrated for the first time a novel mechanism for tumor-host cross-talk mediated by syndecan-1 that is shed from the surface of tumor cells, translocates to the nucleus of bone marrow stromal cells, and inhibits HAT activity. Translocation of the sSDC1 to the nucleus requires the sulfated heparan sulfate chains of the proteoglycan as well as the presence of an unknown molecule(s) bound to those heparan sulfate chains. In addition, we find that an important and highly active heparan sulfate-binding growth factor (*i.e.* HGF) remains bound to the shed syndecan-1 even after its translocation to the nucleus. This indicates that in addition to inhibiting HAT activity, sSDC1 shuttles factors or other heparin-binding molecules to the nucleus.

Inhibition of HAT activity by nuclear sSDC1 could have an important functional impact because histone acetylation and deacetylation regulate chromatin structure (30–32). This is consistent with our finding that the sSDC1 in the nucleus was found in discrete patches located most notably in the euchromatin where there was less DNA staining (Fig. 1*D*). This indicates that sSDC1 is functioning in specific regions of the nucleus where active gene transcription is taking place and

indicates that sSDC1 could impact gene transcription. Ultimately, the regulation of HAT activity by sSDC1 could orchestrate changes in gene expression of host cells resulting in feedback that impacts tumor behavior.

Previous studies have shown that isolated heparan sulfate or heparin can bind HAT enzymes (12, 13); we show here for the first time that this interaction can take place within the nucleus of cells. Importantly, the direct interaction of sSDC1 with HAT enzyme p300 raises the possibility that it is this direct interaction between the two that results in inhibition of HAT activity. However, we cannot rule out the possibility that sSDC1 has other effects on HAT enzymes that decrease their activity.

The role of sSDC1 in the nucleus likely goes beyond its inhibition of HAT activity. Heparan sulfate or heparan sulfate proteoglycan in the nucleus has been demonstrated in multiple cell types where they can regulate cell proliferation and alter transcription factor binding to DNA (33–36). In addition, our finding that sSDC1 can shuttle HGF to the nucleus indicates a role for the proteoglycan in transporting cargo to the nucleus. It has been shown that some heparin-binding growth factors within the nucleus can act to promote cell proliferation and can interact with other nuclear proteins (37, 38). For example, fibroblast growth factor (FGF)-2 in the nucleus binds to casein kinase 2 stimulating its activity, which may lead to an induction of ribosomal gene transcription (38). Other heparin-binding factors such as VEGF have also been detected in the nucleus. During wound healing, accumulation of VEGF in the nucleus correlated with increased protein associated with the coagulation pathway (39). This is interesting because sSDC1 levels also increase during the wound healing process (40), and VEGF may utilize sSDC1 to translocate to the nucleus. HGF function in the nucleus requires further investigation, but it may serve similar functions in controlling cell proliferation and gene expression.

Intact syndecan-1 contains the nuclear localization sequence RMKKK, and mutating this sequence blocked the translocation of full-length syndecan-1 to the nucleus of mesothelioma cells (11). However, the RMKKK sequence is located within the cytoplasmic domain of syndecan-1 and thus is absent in the shed molecule. This indicates that sSDC1 enters the nucleus via an alternative mechanism. Given that removal of heparin-binding molecules from sSDC1 abolishes its translocation to the nucleus, we speculate that one or more of these factors bound to sSDC1 heparan sulfate contain nuclear localization sequences that are responsible for translocation of the complex to the nucleus. In addition to the mechanism mediating translocation to the nucleus, it is likely that the first step in the process is binding of the sSDC1 to the cell surface. This could be due to scavenger receptors at the cell surface that bind to heparan sulfate (24, 41), or it could be through cell surface receptors that bind to their growth factor ligands (*e.g.*, VEGF, FGF) that are complexed with sSDC1. Although it remains unclear, sSDC1 likely enters the cell via receptor-mediated endocytosis following binding to cell surface receptors. This is supported by our finding that treatment of cells with dynasore, an inhibitor that blocks endocytic vesicle formation, inhibits the translocation of sSDC1 to the nucleus,[3] similar to what has been reported for

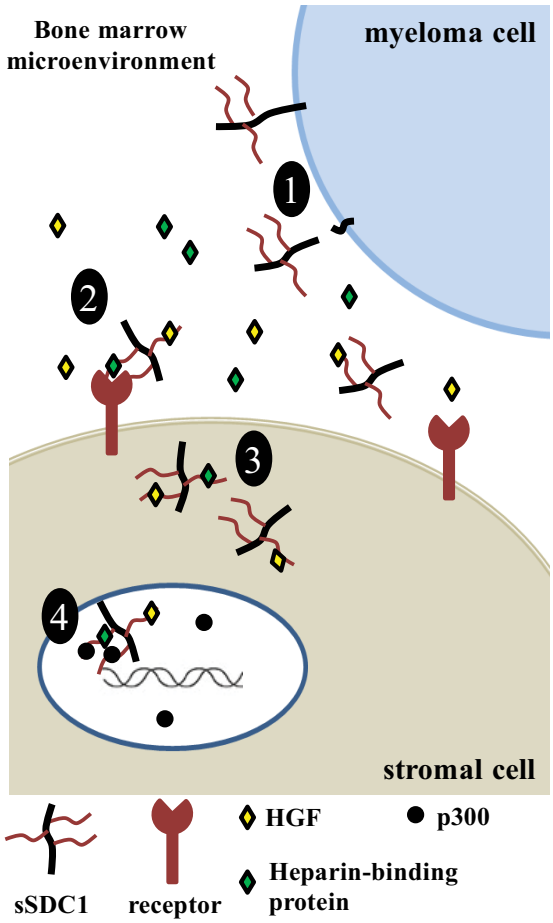

FIGURE 6. **Working model for shed syndecan-1 translocation and functions in the nucleus.** *1*, aggressive myeloma cells shed high levels of SDC1 and secrete heparan sulfate-binding factors (*e.g.* growth factors such as HGF) into the tumor microenvironment. *2*, sSDC1 forms a complex with heparan sulfate-binding factors; the complex binds to the cell surface and enters potentially via an endocytic receptor-mediated pathway. *3*, sSDC1·heparan sulfate-binding protein complex is transported to the nucleus. *4*, in the nucleus, shed syndecan-1 binds p300 and down-regulates histone acetyltransferase activity and histone acetylation. This mechanism for transport of sSDC1 to the nucleus may not be exclusive to sSDC1, but it may also function to transport free heparan sulfate chains or heparanase-generated heparan sulfate fragments to the nucleus.

nuclear translocation of heparin (24). Based on these data, a working model for sSDC1 translocation is shown in Fig. 6. It is likely this translocation mechanism may also occur for free heparan sulfate chains or heparanase-release oligosaccharides that are complexed with heparin-binding proteins.

The finding that sSDC1 translocates to the nucleus and inhibits HAT activation has important implications for myeloma therapy. We have recently demonstrated that in response to some anti-myeloma chemotherapeutic drugs, syndecan-1 shedding is substantially enhanced (9). Translocation of this sSDC1 could impact expression of genes in both tumor and host cells in ways that support tumor survival. In addition, HDAC inhibitors are being used to control tumor progression by promoting acetylation of proteins and gene expression (42). sSDC1 thus might counteract the effects of HDAC inhibitors reducing their antitumor efficacy. These results underscore the importance of gaining a clear understanding of mechanisms regulating sSDC1 translocation to the nucleus and the resulting effects on tumor growth and progression.

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Note Added in Proof—Figs. 1*D* and 3*B* have been modified relative to the versions published on November 17, 2014, as a Paper in Press. In Fig. 1*D*, the white border of the inset has been enlarged and marked with an asterisk to clarify that the inset represents an enlarged section of the same panel. In Fig. 3*B*, the panels labeled "Control" and "sSDC1" that contained the same images as the corresponding panels of Fig. 1*D* in the Paper in Press have been replaced by different images.

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